REMARKS

Applicants have received and reviewed an Office Action dated July 27, 2001. Not to acquiesce to the Examiner's rejections, but to more fully clarify the invention, Applicants have cancelled claims 1-10 and 12-23 and submitted new claims 24-41. Claims 24-41 are now pending. Applicants submit the newly presented claims are supported by the specification as filed and make the following remarks in support of allowance of all pending claims.

Rejection under 35 U.S.C. § 103(a)

The Examiner rejected claims 1-10 and 12-23 under 35 U.S.C. § 103(a) as obvious over *Cohli et al.* (Antisense Res. and Dev. 4:19-26, 1994), *Naldini et al.* (Science 272:263-267, 1996), *Hope et al.* (PNAS, 87:7787-7791, 1990) and *Lisziewicz* (WO92/21750, 1992). Applicants respectfully traverse this rejection.

To establish a *prima facie* case of obviousness, the Examiner must show that: 1) there is some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or combine the teachings; 2) there is a reasonable expectation of success; and 3) the prior art references teach or suggest all of the claimed limitations. MPEP § 2142. For the reasons provided herein, Applicants assert that the Examiner has not met this burden.

A. There is no suggestion to combine or modify the teachings of the cited references.

To make a proper rejection based on obviousness, the Examiner must "show that the references either expressly or impliedly suggest the claimed invention or . . .present a convincing line of reasoning as to why the artisan would have found the claimed invention to have been obvious in light of the teachings of the references." *Ex parte Clapp*, 227 U.S.P.Q. 972, 973 (Bd. Pat. App. & Inter. 1985). Applicants respectfully submit that the Examiner has failed to point to any suggestion in any of the cited references that would motivate one of skill in the art to modify or combine the teachings.

Naldini et al. disclose an HIV-based vector that is capable of transducing nondividing cells (see page 264. col. 3). While *Naldini* sought to optimize gene transfer in non-proliferating cells, the present invention seeks to deliver Tat- and Rev-inducible genes by introducing only the

HIV U3 and R regions having Tat-inducible promoter activity and by introducing a hybrid intron comprising SD and SA sites from two different sources to produce therapeutic gene expression only in HIV-infected cells. *Naldini* does not disclose or suggest the benefits of controlling expression via the interaction of these agents within a cell.

Nor does *Cohli et al.* suggest any such combination. *Cohli et al.* teach MoMuLV-derived retroviral vectors engineered to express HIV-1 packaging signal and Rev response element (RRE) as part of the 3' untranslated region of *neo* in either sense or antisense orientation to impart HIV-1 resistance (see page 19, abstract). *Cohli et al.* do not suggest delivering a gene flanked by splice donor and splice acceptor sites for therapeutic purposes, nor do they suggest the desirability of regulating the expression of the gene of interest by using a combination of agents which are capable of binding to the Tat-inducible sequences in the 5' LTR.

Hope et al. does not remedy the complete absence of suggestion or motivation to combine the disclosures. *Hope et al.* disclose a steroid-inducible transactivator created by fusing Rev with the steroid-binding domain of the glucocorticoid receptor (see page 7787, abstract and col.1). This fusion protein was then used to study role of the arginine-rich region in the localization and function of Rev. In contrast, instead of elucidating the mechanism of Rev function, the present invention provides Rev-inducible promoter activity to deliver a nucleotide sequence via a retroviral vector. Nowhere does *Hope* suggest the desirability of such a system.

Finally, *Lisziewicz* discloses retroviral vectors having increased viral titer and protein expression (see page 1, lines 2-5). Because *Lisziewicz* sought to overcome low viral titers and inefficient gene expression by exploiting Rev and RRE (see page 3, lines 14-20), *Lisziewiscz* makes no mention of modifying the promoter to eliminate low-level expression of the gene of interest. There is no suggestion to eliminate gene expression to avoid the immune response to healthy, uninfected cells. In contrast, the present invention accomplishes these objectives.

MPEP § 2142 states "when the motivation to combine the teachings of the references is not immediately apparent, it is the duty of the examiner to explain why the combination of the teachings is proper." Here, the Examiner has not provided such an explanation, but has instead made only conclusory remarks that it would be obvious to one of ordinary skill in the art at the time of filing to combine the references (see page 4, para. 3).

Applicants further assert that the Examiner has impermissibly used the invention as a "template" to reconstruct the present invention. The Federal Circuit has held that "it is

impermissible to use the claimed invention as an instruction manual or "template" to piece together the teachings of the prior art so that the claimed invention is rendered obvious." *In re Fritch*, 972 F.2d 1260 (Fed. Cir. 1992). Here, none of the cited references suggest the advantages of HIV-dependant expression of a gene of interest created via a Tat-inducible sequence and a Rev-RRE interaction-- undectable levels of gene expression in healthy cells not also producing Rev and Tat.

B. One of skill in the art would have no reasonable expectation of success.

None of the cited references suggest that combining the sequences of the invention in the claimed configuration would successfully produce the retroviral vectors disclosed. The present invention provides a novel means to generate vectors that lead to the expression of the gene of interest only in cells that are infected by HIV. This "safety mechanism" provides a basis to deliver the genes prophylactically without signaling targeting by immune system.

One of skill the art, armed with the teachings of *Naldini*, *Hope*, *Cohli* and *Lisziewicz*, would not have a reasonable expectation that creating the claimed combination would function successfully to eliminate the immune clearance problems associated with typical HIV gene delivery. Indeed, *Lisziewicz* reports that simply combining a tat-inducible vector with a rev response element still leads to low-level expression levels of the protective gene (see page 12, lines 29-33). This would lead one of skill in the art to conclude that basal gene expression is unavoidable with HIV vectors using LTR promoters. The vector of the present invention, however, comprised of an HIV U3 and R region having tat-inducible promoter activity, at least one nucleotide sequence, and a polynucleotide response element (PRE) where the nucleotide sequence and the PRE are located within an intron which is flanked by splice donor and splice acceptor sites derived from different retroviruses, leads to undetectable basal transcription activity, which is unexpected and non-obvious.

C. The cited references do not teach every element of the invention as presently claimed.

The present invention relates to non-lentiviral retroviral vectors that comprise Tat-inducible promoter sequences and polynucleotide response elements (PRE) such as RRE. Further, the relative locations of the Tat and Rev inducible sequences of the invention are in the

HIV U3 and R regions of the 5'LTR region of the retroviral vector, and within an intron flanked by a splice donor site and splice acceptor site, respectively.

It was well known before the priority date of the present invention that the lentiviral vectors disclosed in *Naldini et al.* and *Hope et al.* inherently comprise tat and rev sequences unless they have been removed. These disclosures are acknowledged on page 4, lines 7-11 of the application as filed. The retroviral vectors disclosed in *Cohli et al.* and *Lisziewicz* comprise Tat inducible sequences (such as TAR and PRE sequences) such as the Rev responsive sequences (such as RRE). However, the configuration of these sequences in the vectors of the present invention is very different from the configuration of these sequences in the prior art vectors. The configuration of the present invention, not found in the prior art, provides a means to specifically target HIV-infected cells.

Cohli et al.

Cohli et al. describes MoMuLV retroviral vectors engineered to express an HIV-1 packaging signal and Rev response element (RRE). In these vectors, the RRE sequences are expressed under the control of the MoMuLV 5' LTR and the HSV tk-TAR fusion promoters (see page 22, col. 1, para. 3).

In contrast to the presently claimed invention, *Cohli et al.* does not disclose or suggest: 1) a retroviral vector comprising splice donor (SD), RRE and splice acceptor (SA) sites; 2) a nucleotide sequence located between the SD and SA sites; 3) regulation of expression of the nucleotide sequence using a combination of agents which are capable of binding to the Tat inducible sequences in the 5' LTR of the retroviral vector and the polynucleotide response element (PRE); and/or 4) a hybrid intron comprising splice donor and splice acceptor sites derivable from different retroviral vector sources.

Naldini et al. (1996)

Naldini et al. discloses an HIV-derived retroviral vector that is capable of integrating into the genome of nonproliferating cells. Construction of this vector is based upon a three-plasmid expression system, utlizing a separate packaging construct, env-encoding plasmids from VSV or MLV and a transfer vector. Because it is derived from HIV, the resultant vector inherently contains RRE and Tat inducible (Tar) sequences. Page 4, lines 7-18 of the specification as filed

acknowledges that Tat and Rev regulatory proteins allow efficient transcription and cytoplasmic export of full length vector transcripts as described in *Naldini et al.* (see *Naldini*, p. 263, col 3).

However, *Naldini et al.* does not disclose or suggest the regulation of expression of a nucleotide sequence in a non-lentiviral retroviral vector using a combination of agents which bind to HIV U3 and R regions or functional portions thereof having Tat inducible promoter activity, and/or a PRE located within an intron in the vector. Moreover, the *Naldini* reference does not disclose or suggest a hybrid intron comprising a SD and SA site derivable from different sources. In addition, the coding sequence for the reporter gene lies outside of the RRE-containing intron (see *Naldini* Figure 1). Therefore, the Rev/RRE system of *Naldini et al.* is neither used nor proven to manipulate the expression of a therapeutic gene as in the present application.

Hope et al.

Hope et al. teaches a reporter plasmid (pDM128) derived from the env region of HIV-1. Although *Hope et al.* demonstrates the need for a functional PRE (such as an RRE) to regulate the expression of a nucleotide sequence, *Hope et al.* do not disclose or suggest the regulation of expression of a nucleotide sequence using a combination of agents which bind to Tat inducible sequences (such as Tar sequences) located in the 5' LTR region of a vector other than an HIV vector. Nor does it suggest use in the construct of a PRE (such as an RRE) located within an intron. Moreover, because it teaches a plasmid derived from the HIV genome, it is clear that *Hope et al.* does not disclose or suggest a hybrid intron comprising SD and SA sites derivable from different sources.

In summary, *Hope et al.* discloses a plasmid derived from a lentiviral vector comprising SD and SA sites. *Hope et al.* disclose neither a non-lentiviral retroviral vector with the specific regulatory elements in the 5' LTR region, nor the presence of a hybrid intron comprising SD/SA sites from different sources within the vector.

Consequently, one of skill in the art with knowledge of *Hope et al.* would not have been motivated to prepare a retroviral vector as set out in the present invention.

Lisziewicz (WO 92/21750)

Lisziewicz (WO 92/21750) describes vectors producing increased viral titer and protein expression. Higher level viral titer is achieved by increasing the amount of packageable RNA in the cytoplasm by incorporating a Rev/RRE transport element. However, this high level gene expression is achieved through splicing. Page 5, lines 25-27. Thus, the vectors of Lisziewicz require splicing in order to express the therapeutic gene. The present invention does not. Furthermore, Applicant agrees that the *Lisziewicz* vectors can include HIV LTR regions for regulating the expression of a nucleotide sequence in the presence of Tat (see page 12, lies 14-25). However, *Lisziewicz* does not disclose or suggest using a retroviral vector comprising just HIV U3 and R regions or functional portions thereof having Tat inducible promoter activity in conjunction with non-lentiviral splice sites. Although *Lisziewicz* refers to an "intron" and "intron containing RNA" or "an intron of a foreign gene," *Lisziewicz* makes no reference to SD or SA sites in the non-lentiviral retroviral vector. In view of the fact that the description is silent about the possible existence of splice sites, it is clear that there is no disclosure in *Lisziewicz* concerning a hybrid intron comprising a splice donor and splice acceptor site from two different sources.

In summary, although *Lisziewicz* discloses a non-lentiviral retroviral vector with HIV LTR regions, it does not disclose a non-lentiviral retroviral vector with the specific regulatory elements in the 5' LTR region or the presence of hybrid intron with SD and SA sites from different sources within the vector.

Summary

In conclusion, the newly presented claims are non-obvious over *Cohli et al.* (1994), *Naldini et al.* (1996), *Hope et al.* (1990) and *Lisziewicz* (WO 92/21750), both alone and in combination. The Examiner is invited to contact the Applicants' undersigned representative at the telephone number listed below, if the Examiner believes that doing so will expedite the prosecution of the application.

CONCLUSION

In view of the amendments and remarks made herein, it is respectfully submitted that the application is in condition for allowance. Notification to that effect is earnestly requested.

Respectfully submitted,

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